DO PHARMACEUTICALS IN THE ENVIRONMENT POSE A RISK TO WILDLIFE?

SUPPORTING INFORMATION 1 - DEFINITION OF A PHARMACEUTICAL

Pharmaceuticals are defined as substances of synthetic or biological origin used to diagnose, treat, mitigate or prevent disease or to promote well-being. Pharmaceuticals include low molecular weight products (chemicals), higher molecular weight products (biologics and protein drugs) and vaccines. Pharmaceuticals prescribed for human use are most commonly classified by the Anatomical Therapeutic Chemical (ATC) Classification System for humans (World Health Organization [WHO], 2022). Veterinary use drug classification (WHO, 2021) is also based on the principles of this system. However, from an ecotoxicological perspective, environmental fate, exposure and effects of pharmaceuticals are commonly examined based on physicochemical properties, a simplified classification based on their therapeutic properties or mode of action.

SUPPORTING INFORMATION 2 -EXPOSURE PATHWAYS

Figure S2a-d summarize exposure pathways by which wildlife could be exposed to pharmaceuticals. The main source of pharmaceuticals in the environment is believed to be their use in human and animal patients (Daughton & Ternes, 1999). Patients and medicated animals excrete a significant fraction of the administered dose of the pharmaceutical as an active compound (unchanged parent and/or active metabolites). In the case of humans, waste either goes to the sewer or septic system. Once in the sewer, the wastewater is transported to the wastewater treatment plant where exposure of wildlife could result from i) direct foraging on contaminated invertebrates, ii) incomplete removal or discharge of effluent containing pharmaceutical residues to the aquatic environment, iii) use of effluent for irrigation of forests, fields or turf, iv) application of biosolids to farmland as a soil amendment. For veterinary drugs, excretion typically occurs directly by the animals to the field or bedding material. It is possible that wildlife could be exposed by directly foraging on the backs of cattle and other animals that have been ‘dipped’ (this also expands the definition of ‘pharmaceutical’ as there are pesticides used as dips) and it is well known that the Asian (*Gyps*) vulture crisis resulted from exposure to diclofenac that was ingested in the tissues of ungulate carcasses that were medicated shortly before death. In some cultures, livestock carcasses, and even human remains (e.g., Parsi sky burial towers) are intentionally left out for scavengers. Exposure of scavengers to barbiturates via consumption of companion animal carcasses that had been euthanized and

placed at dumps were documented in the 1980s in North America. Today, barbiturate poisoning is still documented in some regions (e.g., Spain, France), although some may be intentional.

A small amount of emissions during the manufacturing process will likely also contribute to pharmaceutical residues in the environment, although it is not thought to be a significant source of exposure to wildlife compared to other sources. Inappropriate medication disposal to landfill or down the drain to sewerage and septic systems rather than using takeback schemes could lead to exposure of wildlife via direct foraging on landfills or trophic transfer when runoff and leachate enter aquatic environments. Other hypothetical exposure pathways include leakage from septic systems, and seepage and runoff of antibiotics from feed lots that contained medicated diets.

The best-known exposure pathways are those related to scavengers (diclofenac and other non-steroidal anti-inflammatory drug (non-steroidal anti-inflammatory drugs [NSAIDs], barbiturates), with limited data on the significance of wastewater and landfills as pharmaceutical exposure routes for wildlife.

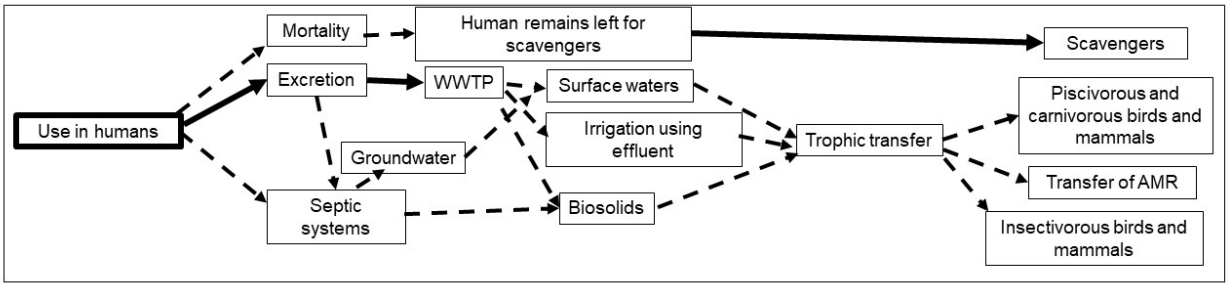

Figure S2a: Known (solid arrows) and hypothetical (dashed arrows) pharmaceutical exposure pathways for wildlife from use in humans. WWTP =Wastewater Treatment Plant; AMR = Antimicrobial Resistance

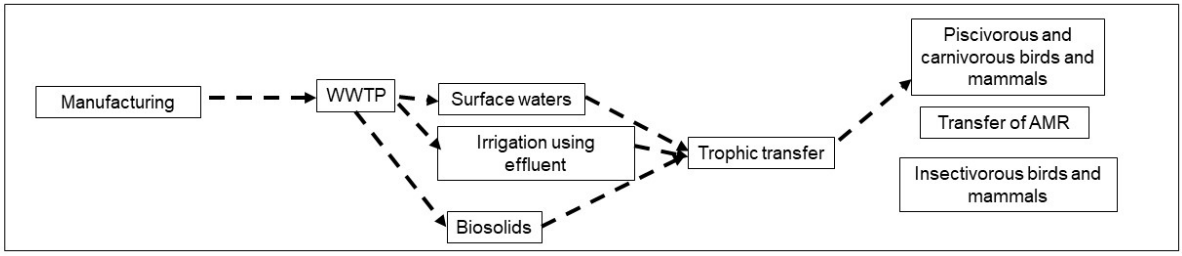

Figure S2b: Known (solid arrows) and hypothetical (dashed arrows) pharmaceutical exposure pathways for wildlife from manufacturing. WWTP =Wastewater Treatment Plant; AMR = Antimicrobial Resistance

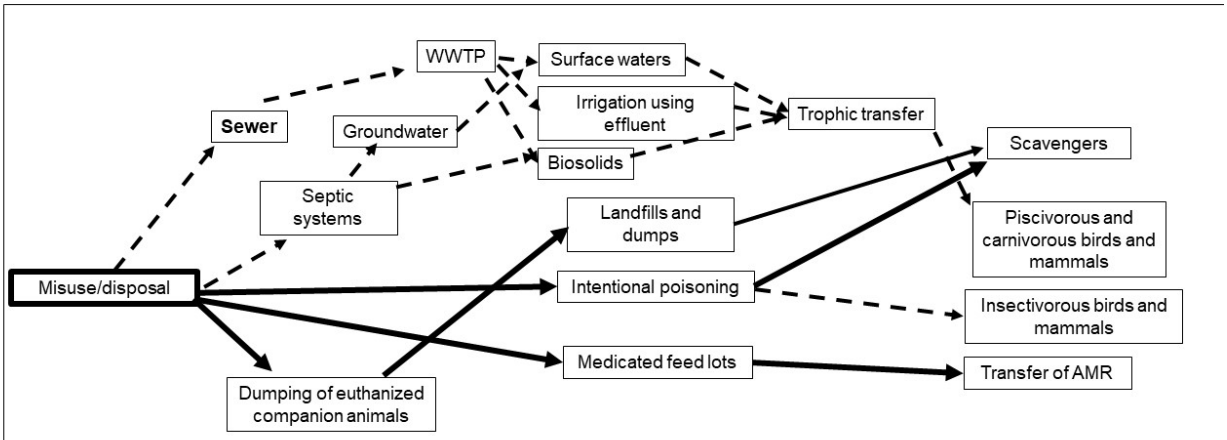

Figure S2c: Known (solid arrows) and hypothetical (dashed arrows) pharmaceutical exposure pathways for wildlife from misuse/disposal. WWTP =Wastewater Treatment Plant; AMR = Antimicrobial Resistance

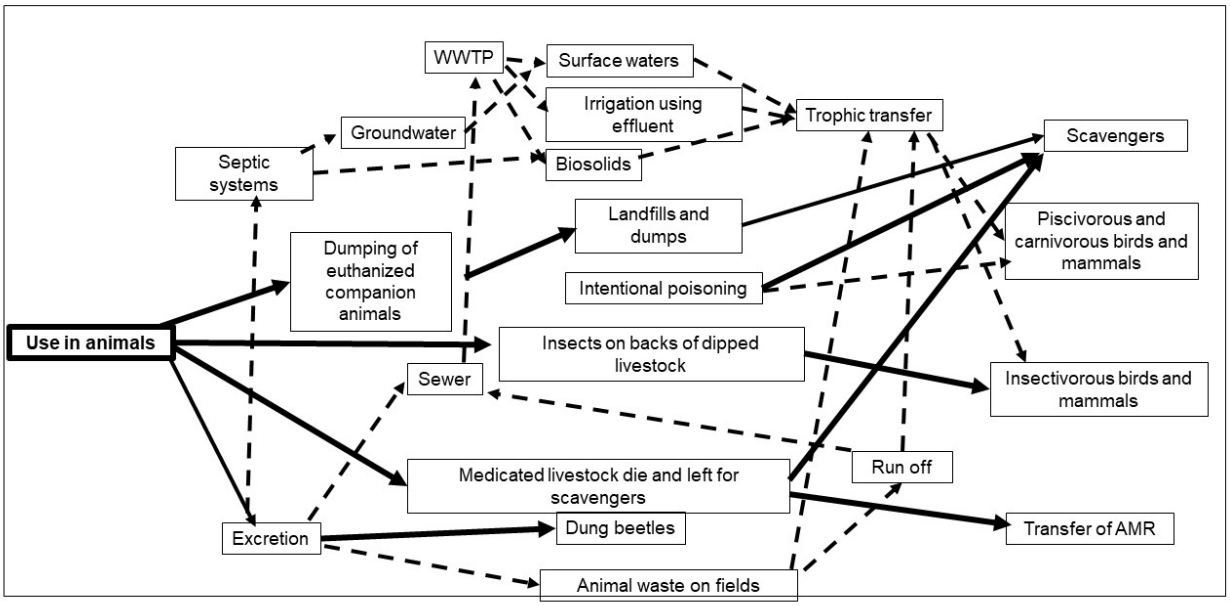

Figure S2d: Known (solid arrows) and hypothetical (dashed arrows) pharmaceutical exposure pathways for wildlife from use in animals. WWTP =Wastewater Treatment Plant; AMR = Antimicrobial Resistance

SUPPORTING INFORMATION 3 - GEOGRAPHICALLY PATCHY DATA FOR EXPOSURE, HAZARD AND RISK OF PHARMACEUTICALS FOR WILDLIFE

A bias toward research in developed countries serves to emphasize associations between surface water contamination and illicit drug usage, but largely ignores potential issues in less developed countries, which are often key areas of drug production, and typically have limited wastewater treatment (Rosi-Marshall et al., 2015). Indeed, the geographically patchy data on pharmaceuticals is not isolated to illicit drugs. To date, the majority of research on pharmaceutical exposure of wildlife has focused on areas in South Asia and South Africa, related to the *Gyps* vulture crisis. In 2014, Kookana et al. (2014) highlighted the importance of gaining an understanding of pharmaceutical contamination in lower and middle-income countries. In such countries, exposure pathways and contamination are likely quite different compared to high income countries (e.g., lower- and middle- income countries typically have less stringent regulations for minimizing environmental contamination, sewerage systems are less well developed likely resulting in seepage into the environment, increased production of pharmaceuticals in these regions as companies seek out lower manufacturing costs). Indeed, a comprehensive study examining pharmaceutical contamination of rivers across 104 countries (including 36 countries with no previous data on pharmaceutical contamination) (Wilkinson et al., 2022) found that the rivers with highest

pharmaceutical contamination were in lower- and middle- income countries in South America, Sub-Saharan Africa and South Asia.

SUPPORTING INFORMATION 4 - GUIDANCE FOR INDUSTRY

Guidance for industry for registration of human medicines are provided by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) (FDA, 1998a; FDA, 1998b; EMA, 2006; EMA, 2016) while for veterinary medicines, there has been harmonized guidance across the European Union, Japan and USA since the early 2000s. Both schemes are similar with phased or tiered approaches initially screening out drugs that are not used in high volumes before requiring acute and chronic tests in aquatic and terrestrial species that follow Organisation for Economic Co-operation and Development (OECD) or the U.S. Environmental Protection Agency (US EPA)'s Office of Chemical Safety and Pollution Prevention (OCSPP) guidelines with the assessment [safety] factors reduced when chronic endpoints become available (see Supporting Information for a detailed discussion and Figure 1 in the manuscript for a simplified schematic). Notably, no specific tests in wildlife are routinely required, although extensive mammalian data are available from pre-clinical trials. The need for tests in birds and mammals is considered on a case-by-case basis (e.g., if exposure and acute toxicity are possible). An example of a pharmaceutical in this category is the NSAID flunixin when submitted for registration by the FDA in the United States. In this case, flunixin was being registered as a pour over formulation for cattle (FDA, 2017b). That said, the Asian vulture crisis (Oaks et al., 2004) was unlikely to have been predicted even if standard registration studies in northern bobwhite, mallard and passerines had been available for diclofenac due to the differential sensitivity even among vultures (e.g., compare the median lethal dose (LD_{50}) in Swan et al., 2006; Rattner et al., 2008; also see Duncan et al., 2018 and Hasan et al., 2018) and the societal factors that played a role in the creating this unique exposure route (see NSAIDs and Scavengers section in the manuscript).

SI 4.1 Veterinary drugs

The goals are to provide protection at the ecosystem level, yet in some circumstances it is acknowledged that the product may be a significant concern for individuals. There is a tiered approach consisting of 3 phases (phase I, phase IIa and IIb), a screening step is done in phase I (Veterinary International Conference on Harmonization [VICH] guidance document 89, VICH, 2000) while phase II (VICH guidance document 166, VICH, 2006) involves actual tests with generally acute tests performed in phase

IIa and chronic tests performed if needed in phase IIb. The hazard data are combined with predicted environmental concentrations to evaluate risk.

The phase I screening level assessment is essentially a decision tree that the registrant follows until "Yes" can be answered for one of the questions (e.g., Will the Veterinary Medicinal Product be used only in non-food animals? Is the Veterinary Medicinal Product extensively metabolized in the treated animals? Is entry to the environment prevented through disposal of the terrestrial or aquatic waste matrix?) (VICH, 2000). Upon a "yes' response, no further assessment is required. If the predicted environmental concentration (based on usage and environmental fate) of a veterinary drug is below 1 µg/L for aquatic environments or less than 100 µg/kg soil for terrestrial environments, then no further experimental testing is required.

Phase IIa uses experimental hazard data for aquatic and terrestrial species (VICH, 2006). The tests in phase IIa are generally acute tests following OECD test guidelines (e.g., algal growth inhibition, fish and daphnia, soil microorganisms, earthworms and non-target terrestrial plants). Typically, LD_{50}s or median effective concentrations (EC_{50}s) are used are used in conjunction with species-specific assessment factor, ranging from 10-1,000. For the exposure component, 100% excretion as parent is assumed, with an assessment factor of 10 for earthworm, 100 for fish and plants, and 1,000 for all other species being applied. If a risk quotient [RQ = (hazard/assessment factor)/predicted exposure] is >1, then a refinement of the Predicted Environmental Concentration [PEC] that accounts for patient metabolism of the drug is included; if the RQ is still >1, then further phase IIb testing is required.

In Phase IIb, chronic tests are usually conducted in the same species as the acute tests in Phase IIa, or additional non-target terrestrial plant species are used. The risk assessment uses the no-observed effect concentration (NOEC) and the assessment factor is typically 10. If the risk quotient is still ≥1, then the registrant must seek regulatory guidance. Phase IIb may require a fish bioconcentration factor (BCF) study if the log of the octanol-water partition coefficient (kow) of the active ingredient is ≥4.

It is notable from the VICH Phase II guidance that no tests in birds or mammals are routinely required for the registration of a veterinary drug. The need for tests in birds and mammals is considered on a case-by-case basis (e.g., if exposure and acute toxicity are possible).

SI 4.2 Human use pharmaceuticals

For human use pharmaceuticals, there is no component of the risk assessment that involves tests in wildlife, although there is usually a significant amount of in vivo mammalian testing conducted to support the product registration e.g., rat, mouse, dog (FDA, 1998a; FDA, 1998b).

In the United States, no ecological risk assessment (ERA) is required if annual production is less than approximately 46,000 kg/year (i.e., this is expected to be equivalent to <1 ppb in aquatic environment at the point of entry), (Crawley, 2020). A tiered approach is required if concentrations in the aquatic environment will be >1 ppb at the point of entry. In the European Union (EU), risk assessment involves Organisation for Economic Co-operation and Development OECD guideline studies, which could also be used for the United States (European Medicines Agency [EMA], 2006; EMA, 2016). In total, it takes about 2 years to generate the ERA package (Crawley, 2020; EMA, 2006; EMA, 2016).

In Europe, the drug is screened in Phase I of the ERA for persistence, bioaccumulation and toxicity by examining physicochemical properties and epidemiologic data. The PEC in surface water is calculated with Phase II triggered if the value is greater than 0.01 ug/L (i.e., 10 ppb). Phase IIa includes environmental fate studies and aquatic toxicity, with tests including algae, daphnia, fish early life stage and activated sludge respiration. In Phase IIb, further testing may be required such as estimation of bioconcentration factor or bioaccumulation in fish, terrestrial earthworm acute, non-target terrestrial plants, soil microbial tests, non-target terrestrial plants and Collembola reproduction. Higher tier studies may be required if endocrine activity is suspected (Fish Short Term Reproduction Assay, Fish Full Life Cycle Test, Medaka Extended One Generation Reproduction Test).

In the United States, the ecotoxicity tests required for pharmaceutical risk assessment are divided among 3 tiers with the assessment factor reduced by an order of magnitude with each tier, starting at 1,000 in tier 1 and going down to 10 in tier 3. In tier 1, at least one test is required (acute fish, acute aquatic invertebrate, algal growth inhibition, non-target terrestrial plants, earthworm acute or effects on soil microbiota). In tier 2, all 6 tests are usually required, while tier 3 would include chronic toxicity testing.

SI 4.3 Regulatory range of uses approved and potential off label use

In Europe, pharmaceuticals are regulated by a centralized authority, the European Medicines Agency (EMA) for which the European Food Safety Authority (EFSA) provides guidance for each member state. Even so, each country has their own product labels and specific regulations for different active ingredients, always within European established limits. This regulation is focused on maximum residue limits (MRL) of pharmaceuticals used in veterinary medicine that must not be exceeded in products destined for human consumption (EU, 37/2010; EC, 2010). There are no regulations for carcass removal of domestic animals that are not destined for human consumption; these carcasses can enter wildlife food webs following inappropriate disposal. In some cases, the specific mitigation measures to avoid wildlife pharmaceutical exposure are just recommendations in the prospect leaflet of the product (e.g., diclofenac and pentobarbital in Spain). In terms of residues of human use pharmaceuticals, there are several pathways (i.e., wastewater treatment plants, hospital discharge) that release these products, mainly into aquatic ecosystems, and for

which there is no regulatory concentration limit in the environment (Boxall et al., 2012). Moreover, environmental risk assessments proposed to avoid the presence of these substances in the environment still do not consider all specific exposure routes to scavengers or wildlife in general (EMA, 2018: Fabrega & Carapeto, 2020).

SUPPORTING INFORMATION 5 -DEFINITION OF WILDLIFE

The definition of wildlife varies across sources as to whether fish and insects are included (e.g., Merriam-Webster English dictionary https://www.merriam-webster.com/dictionary/wildlife compared with Oxford English dictionary https://www.oxfordlearnersdictionaries.com/definition/english/wildlife).

SUPPORTING INFORMATION 6 VULTURES AND NSAIDS

SI 6.1 Vulture population recovery

In 2019, populations of the Indian vulture (*Gyps indicus*), white-rumped vulture (*Gyps bengalensis*) and the slender-billed vulture (*Gyps tenuirostris*) were estimated to be as low as 12,000, 6,000 and 1,000 individuals respectively (GK Today, 2019), when decades ago numbers of these species in India had been in the millions. As a result of the vulture population declines, the government of India established eight vulture conservation breeding centers and the conservation status of the aforementioned has been upgraded from Schedule IV to Schedule I of the 1972 Wildlife (Protection) Act (Parliament of India, 1972). The conservation program in India has by and large been meaningful and satisfactory, with some birds released to the wild (Personal Communication, S. Muralidharan, Salim Ali Centre for Ornithology & Natural History, 5th November, 2021).

SI 6.2 Differential metabolism of NSAIDs in birds

For pharmacokinetics (i.e., absorption, distribution, metabolism and excretion), metabolism is most commonly the factor associated with a difference in drug effect (Toutain & Bousquet-Mélou, 2005). While metabolism can occur in any tissue, liver is predominantly the site of metabolism. Drug metabolism is characterized as a two-stage process, with phase I producing more polar metabolites and often being facilitated by the cytochrome associated enzymes (e.g., mono-oxygenases, epoxides), and phase II involving enzymatic conjugation which increases water solubility. Since metabolism is enzymatic, this is generally the major point for interspecies differences, with variation seen in the actual enzyme type present, ratio of enzymes or overall enzyme activities (Fink-Gremmels, 2008). Unfortunately, the complexity of these metabolic differences means they are difficult to characterize

without detailed *in vitro* or *in vivo* studies. To illustrate this concept, one can examine the NSAID ketoprofen that has been commonly used for pain management in raptors. Ketoprofen proved to be toxic to vultures (Naidoo et al., 2010). When evaluating the pharmacokinetics of ketoprofen in vultures, there is clear evidence of metabolic constraint with birds that succumbed to toxicity having an unexpected long elimination half-life compared to birds that survived exposure. While the exact mechanism of toxicity (perhaps enzyme deficiency) in vultures has yet to be elucidated, it is likely at the activity level of CYP2C8/9/18 which has been identified as a point of concern in drug toxicities in susceptible humans (Yasar, 2001). While the names of the enzymes differ between species (naming convention not standardized), the enzymes do have commonality in their binding sites (enzymes with similar binding sites metabolize similar substrates).

SUPPORTING INFORMATION 7 - FLUOXETINE AND STARLINGS

One body of work investigating the potential hazard of the human use pharmaceutical fluoxetine (selective serotonin reuptake inhibitor antidepressant) in wastewater treatment plants (WWTP) has examined potential effects of environmentally relevant exposures to a passerine bird, the European starling *Sturnus vulgaris* (Bean et al., 2014; Bean et al., 2017; Whitlock et al., 2018; Whitlock et al., 2019). Controlled studies designed to simulate avian exposures via invertebrates at WWTP trickling filter beds found indications that predicted environmentally realistic concentrations administered via spiked invertebrates for ~6 months may cause subtle effects on foraging (Bean et al., 2014) and courtship behavior (Whitlock et al. 2019). Starlings are red listed in the United Kingdom (UK) due to population declines (89% between 1967 and 2018; Woodward et al., 2020) related to survival of first year birds, likely due to limited availability of food supply in the autumn (BTO, 2002). At present, the importance of the exposure pathway (i.e., birds eating fluoxetine contaminated invertebrates from WWTPs) and the biological significance of fluoxetine effects is still a knowledge gap, i.e., do they translate from the laboratory to the field and behavior as a relevant apical endpoints. For example, it is possible that exposures in these experiments were overly conservative (worst case). The initial experiments of Bean et al. (2014; 2017) based the predicted environmentally relevant dose administered to the starlings on several factors: i) fluoxetine concentration in invertebrates was calculated based on its usage in England, ii) percentage of the dose human patients typically excrete as parent compound, iii) dilution in wastewater and bioaccumulation in an invertebrate and iv) that 50% of a free-ranging starling's invertebrate prey would come from the wastewater treatment plant trickling filter bed (daily dose of 0.92 µg/bird/d). More

recent experiments (Whitlock et al., 2018; Whitlock et al., 2019) assumed a worst-case exposure scenario, with 100% of a starling's invertebrate prey coming from WWTP trickling filter beds and the highest concentration of fluoxetine detected in earthworms collected from the four UK WWTPs. The experiments of Whitlock et al. (2018; 2019) used a daily dose of 2.7 µg/bird/d. The foraging percentages and consumption rates used for the exposure calculations were based upon UK field observations from the late 1970s (Fuller & Glue, 1978) so it remains unverified as to whether the many changes to the landscape and ecosystems mean that trickling filter beds are currently as important to foraging birds as they were 44 years ago. Therefore, the importance of this exposure route and risk remain to be determined.

SUPPORTING INFORMATION 8 - TROPHIC TRANSFER OF PHARMACEUTICALS TO OSPREYS

Ospreys (*Pandion haliaetus*) are a high trophic level species that are strictly piscivorous, and have been used as sentinels of environmental contamination and change in many settings (Grove et al., 2009). In Chesapeake Bay, the greatest diltiazem concentration in osprey plasma was 28% of the Human Therapeutic Plasma Concentration [HTC], while greatest nestling plasma acetaminophen and diclofenac concentrations were 2 to 3 orders of magnitude below the HTC. It was suggested that if the theoretical elimination half-life required for ospreys and perhaps other wildlife was shorter than the known active pharmaceutical ingredient (API) elimination rate in humans, then it would not be unreasonable to assume that APIs might be accumulated and potentially exert effects within an ecologically relevant time frame (Bean & Rattner, 2018). Modeling of trophic transfer data was used to further examine this hypothesis. Notably, API surface water concentration from the Delaware study region and theoretical bioconcentration factors at pH 8 in fish did not predict measured concentration in fish (Bean et al., 2018). Moreover, using the greatest concentration of each API detected in fish plasma from this region, the predicted maximum concentration in osprey nestling plasma after a meal was <1 ng/mL, which corresponds to at least 2 orders of magnitude below the HTC. The one exception to this prediction was for diclofenac, with a predicted concentration of 15.5 ng/mL in nestling plasma. Overall, these data and predictions indicate that the risk of therapeutic or toxicological effects associated with trophic transfer of APIs and metabolites to osprey nestlings in the Chesapeake and Delaware Bay regions is seemingly low.

SUPPORTING INFORMATION 9 – NON-INVASIVE METHODS FOR EXPOSURE ASSESSMENT

Table S9: Non-invasive sampling (feathers, hair, carcasses) for monitoring exposure to pharmaceutical residues. NSAID = non-steroidal anti-inflammatory drug; UK = United Kingdom; NGO = non-governmental organization.

Non-invasive exposure assessment	Pharmaceutical	Species	Evidence	Reference
Feathers	Diclofenac, ibuprofen, naproxen, nimesulide	Gulls and terns	High prevalence of diclofenac has been found in feathers used for non-invasive sampling of gulls and terns (100% and 83%, respectively)	Distefano et al., 2022
Feathers	Citalopram, N-desmethylcitalopram, fluoxetine, fluvoxamine, sertraline, and venlafaxine	Waterbirds	Other pharmaceuticals used as antidepressants in human medicine were detected during this monitoring in waterbirds	Distefano et al., 2022
Feathers	Fluoxetine	European starlings	Captive Eurasian starlings exposed to fluoxetine (0.035 mg/kg) for 28 days showed averaged 11.4 ng/g in feathers grown during exposure, which is much lower than concentrations detected in their tissues (up to 111.2 ng/g liver). Interestingly, starlings also showed up to 27.0 ng/g of fluoxetine in feathers grown while in the wild, although also see discussion in supporting information Section V	Whitlock et al., 2019
Feathers	Oxytetracycline, lincomycin	Chicken	The relationship between levels in feathers and tissues has been studied for some antibiotics (i.e., oxytetracycline, lincomycin) in experimentally exposed chicken	Cornejo et al., 2017; Pokrant et al., 2019

Non-invasive exposure assessment	Pharmaceutical	Species	Evidence	Reference
Hair	Diclofenac, ibuprofen	Otters	In aquatic ecosystems, some NSAIDs (i.e., ibuprofen, diclofenac) have been detected in up to 53.6% of hair samples of Eurasian otters from the UK	Richards et al., 2011
Livestock carcasses	Diclofenac and other NSAIDs	Ungulate carcasses	The presence of diclofenac in ungulate carcasses has been related to the severe population declines of Asian *Gyps* vultures. Diclofenac prevalence in these carcasses from India reached up to 11.1-13.9%, and in addition other NSAIDs were detected domestic livestock tissues	Taggart et al., 2007a; 2007b; 2009
Livestock carcasses	Diclofenac, ketoprofen, meloxicam	Pig, sheep	In Spain, NSAID prevalence in carrion supplied at feeding stations for vultures was 3.07%, pig and sheep tissues analysed showed residues of flunixin (1.28%), diclofenac, ketoprofen and meloxicam (0.64%, each)	Herrero-Villar et al., 2020
Livestock carcasses	Pentobarbital	Ungulate	Barbiturates have been detected in carcasses available to avian scavengers, and directly linked to a large poisoning event affecting griffon vultures	Herrero-Villar et al., 2021
Livestock carcasses	Oxytetracycline, trimethoprim, sulfadiazine, penicillin G, ciprofloxacin, enrofloxacin, tetracycline	Ungulate	Antibiotics have also been reported in ungulate carcasses supplied at vulture feeding stations in Portugal, even though these have not yet been found to cause acute toxicity to avian scavengers	Gómez-Ramírez et al., 2018
Wildlife incident reports	NA	cetaceans, predatory birds, and otters	WILDCOMS network UK monitor disease and contaminants in vertebrates found dead	www.wildcoms.org.uk

Non-invasive exposure assessment	**Pharmaceutical**	**Species**	**Evidence**	**Reference**
Wildlife incident reports	NA	All	SAGIR network for the monitoring of wildlife mortalities in France with an implication of the hunters and research laboratories, Acute poisoning of Red Kites (Milvus milvus) in France: data from the Sagir network	Berny & Gaillet 2008
Wildlife incident reports	NA	All	ANTIDOTO network of NGOs for the monitoring of wildlife mortality in Spain with the implication of public administrations, police and research labs	https://www.venenono.org/?page_id=286
			Direct evidence of poison-driven widespread population decline in a wild vertebrate.	Mateo- Tomás et al., 2020
			Relationship of the toxicity of pesticide formulations and their commercial restrictions with the frequency of animal poisonings.	Martinez-Haro et al., 2008
			Use of poisoned baits against wildlife. A retrospective 17-year study in the natural environment of Extremadura (Spain).	Ibáñez-Pernía et al., 2022
			Developing a European network of analytical laboratories and government institutions to prevent poisoning of raptors.	Valverde et al., 2022
			Evidence of avian and mammalian scavengers poisoned by barbiturates	Herrero-Villar et al., 2021

SUPPORTING INFORMATION 10 - EFFECTS ASSESSMENT

SI 10.1 New Approach Methods

What is our current understanding of the topic? With the pharmacokinetic and pharmacodynamic mechanisms underlying toxicity being unknown in many wild species, a form of predictive toxicology would be desirable to elucidate the likely toxic potential of chemicals in different species as part of their toxicity assessment. To place this into perspective, most studies are undertaken in rodents with a safety factor of 10 applied to extrapolate to human safety (Hartung, 2009; Spurgeon et al., 2020). While this convention is useful, it is not very predictive for the multitude of wild species and fails to consider that some species would be highly sensitive due pharmacokinetic or pharmacodynamic differences. Starting as early as the 1960s (although chemists were making predictions based on chemical structures as early as 1816), simulations based on regression models have been developed known as QSARs (quantitative structure-activity relationships) (Dearden, 2016; Raies et al., 2016). The aim of such predictions is to determine the structural relationship between a molecule and its known in vivo toxicity. This subsequently allows for predictions of the toxicity of similar molecules. As more information is inputted into these models, better predictions can be made across different chemical classes and species.

While such models are extremely useful, they do have restrictions in that a training data set still needs to be generated. When dealing with a wild species, this information is usually not known and in most cases is difficult to generate due to the status (threatened or endangered) of many species in question. Using the NSAID toxicity in vultures as an example, despite data on a number of toxic drugs being available, toxicity of the remaining NSAIDs remains difficult to predict due to the structural diversity of this group.

Another way to overcome this diversity is by genomic and transcriptome analysis (Panahi et al., 2018). Fully developed phylogenetic trees based on receptor similarities may allow for the grouping of species to provide an idea of potential surrogate (model) species that may be used for toxicity studies or to identify other susceptible species (Adawaren et al., 2020). Several prominent programs have now been developed which allow quantification of the structure and function of the genome, and changes (e.g., gene expression) correlated with exposure to environmental pressures, including toxicants. Efforts are now being made to integrate such toxicogenomic approaches into regulatory frameworks such as REACH (Kinaret et al., 2020). Hepatic and renal transcriptome analysis can also be very useful in predicting the metabolic enzymes present in the species, which in combination with *in silico* metabolic predictor tools, could allow one to ascertain which molecules would be metabolized slowly (Rydberg et al., 2010; Banerjee et al., 2020). It has also been suggested that primary cell cultures can assist in vitro metabolic

studies. However, it should be noted that these tools are only aides in predicting toxicity, and that in vivo toxicity studies may still be required in the species predicted to be susceptible.

For avian species, an additional model that can be considered, is use of embryonated eggs for toxicity testing (Nishigori et al., 1992). Furthermore, it may be possible to use the commonly available chicken embryo for such studies. A major advantage of the model is that early-stage embryos have limited metabolic capacity (relative to late-stage embryos and beyond) and thus might provide a sensitive screen for potential effects of a drug. Unfortunately, due to the enclosed nature of the egg, the risk is that the drug may be incorrectly classified as highly toxic due to it being un-metabolized with subsequent long-term exposure of cells, which would not happen in vivo.

SI 10.2 Potential for unintended sublethal effects of pharmaceuticals in non-target wildlife

While in some instances, pharmaceutical exposure of non-target wildlife has lethal consequences (e.g., livestock uses of diclofenac poisoning vultures in Section II and barbiturates in euthanized animal carcasses in Section III (Thomas, 1999) and organophosphorous pesticides used in livestock dips killing various species of birds in Section I of the manuscript), it is likely that low-level exposure to some pharmaceuticals would cause sublethal effects, followed by recovery. Theoretically, as part of the sequelae of temporary intoxication, low- level exposure to diclofenac exposure might compromise renal function in scavenging birds resulting in temporary uremia (Oaks et al., 2004), barbiturate exposure could affect behavior (Gonzalez-Jassi et al., 2022), and thermoregulatory function might be impaired by organophosphorus compounds (Rattner et al. 1984; Rattner et al., 1987). While such sublethal effects seem to be logical consequences of low-level exposure, and documentation of such responses in free-ranging wildlife might appear in case records of intoxicated animals undergoing rehabilitation, formal description in the peer-reviewed scientific literature is lacking as most of the focus is on wildlife carcasses and cause of death determinations (e.g., Herrero-Villar et al., 2021).

SI 10.3 Indirect/ food web effects

What is our current understanding of the topic? Part of the adverse effects on wildlife species from the use of a chemical substance and their release into the environment is not explained by its direct toxicity, but is caused by its non-direct effects on other species (i.e., plants, insects) on which it depends (i.e., food, habitat) (Fleeger et al., 2003). The effects of chemical substances on food webs and species assemblages has been studied for some chemicals in a few ecosystems (e.g., pesticides in terrestrial ecosystems (Sotherton & Holland, 2002, Kraus et al., 2021) or pharmaceuticals in aquatic ecosystems) (Van de Perre et al., 2022), but there are still some important gaps in this field. The effect of veterinary

antiparasitics on dung beetle communities is one example of the disturbance that can be caused by pharmaceuticals on key species that can lead to ecological dysfunctions with consequences on many other species (Tonelli et al., 2020).

What are the future research priorities? Developing an understanding of the effects of pharmaceuticals on biodiversity, and the implications for wildlife populations and their distribution, is an emerging field of research. Impacts of pharmaceuticals on the abundance on prey items such as insects in aquatic and terrestrial environments might be one area to focus on initially and evaluate how this relates with geospatial data of wildlife populations.

10.4 Illegal use of drugs to deliberately poison wildlife

What is our current knowledge of the topic? Alarmingly, there have also been recent reports of a few cases of carcasses being baited with phenobarbital (used in veterinary medicine for epileptic seizure treatment) to illegally kill predators (Herrero-Villar et al., 2021), and baits with acetaminophen to kill feral cats in urban areas are commonly detected in the forensic toxicology laboratories (e.g., in the Laboratory of Wildlife Toxicology at IREC; R. Mateo, pers. comm.). In other areas, wildlife may also be harvested for use in traditional medicines e.g., Mashele et al. (2021).

What are the future research priorities?

- Conduct carcass surveys in regions where it is suspected that predators and scavengers are intentionally being poisoned to identify baiting with barbiturates and other drugs
- Determine the extent to which wildlife harvested for traditional medicines is impacting populations and for endangered species, the extent to which individuals are impacted

10.5 Illicit drugs

On a related theme, the use of illicit drugs by humans could also lead to exposure of wildlife. Such drugs may be highly potent, and thus would have the potential for effects at low concentrations. Occurrence of illicit drugs (and their metabolites) in the environment has largely been found on analysis of wastewaters, and reviews suggest that contamination of surface waters is a global issue (e.g., Chen et al., 2021; Rosi-Marshall et al., 2015). Reports of occurrence and effects in wildlife are increasing, and potential impacts (although focused primarily on invertebrates and fish) are diverse, including cellular-, immune- and geno-toxicity (Chen et al. 2021; Rosi-Marshall et al., 2015). Potential effects on the structure and function of aquatic communities are also described (e.g., Lee et al., 2016; Maasz et al., 2020).

What are the future research priorities? Evaluation of the importance of exposure to illicit drug residues in the environment for wildlife

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